RESEARCH PAPER

Household-Scale Bioethanol Recovery from Mixed Fruit Peel Waste (Watermelon, Muskmelon, Strawberry, Tomato and Lemon) via Sequential Self-Fermentation and Indigenous Raisin-Yeast Re-Fermentation: A Spectroscopic and Third-Party Certified Pilot Study

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ABSTRACT

Domestic fruit-peel waste offers a readily available, non-food feedstock for decentralized bioethanol production. Building on an earlier pilot study, this study investigated a two-stage fermentation process using a 2.0 kg composite of watermelon, muskmelon, strawberry, tomato, and lemon peels collected from a household in Faridabad, Haryana, India. Chopped peels underwent spontaneous fermentation for 7 days under household conditions, with daytime ambient temperatures of approximately 40–46°C, relying on native peel-associated microbiota without added yeast, acid treatment, or thermal pretreatment. The filtered broth was subsequently inoculated with an indigenous, raisin-derived yeast starter cultivated for 72 h and fermented for another 7 days at 35–37°C in the dark. Ethanol content was quantified by gas chromatography at the FICCI Research & Analysis Centre, New Delhi, while Fourier-transform infrared (FTIR) and nuclear magnetic resonance (NMR) spectroscopy provided complementary characterization. Ethanol content increased from 0.15% in the pre-fermentation baseline sample to 1.04% after primary fermentation and 6.38% after secondary fermentation, representing an approximately 6.1-fold increase relative to the primary-stage broth. FTIR analysis identified hydroxyl stretching vibrations, while NMR signals were consistent with the ethyl-group structure of ethanol. These preliminary findings demonstrate increased ethanol accumulation during sequential spontaneous and starter-inoculated fermentation of mixed fruit-peel waste without acid or thermal pretreatment. However, the contribution of yeast supplementation cannot be distinguished from that of the additional fermentation period without a time-matched uninoculated control. Replicated, controlled studies, together with ethanol recovery and economic assessments, are required to establish the reproducibility and practical feasibility of this approach for decentralized waste-to-energy applications.

Keywords: Bioethanol; fruit peel waste; indigenous yeast; self-fermentation; re-fermentation; Saccharomyces; FTIR spectroscopy; NMR spectroscopy; circular economy; decentralized waste-to-energy.

How to cite this article: Vishakha Tiwari1 Ashish Srivastava2 Shabnam Khatoon1 Nisha Saxena3 Natwar Kumar Jha3 Poonam Shukla1*. Household-Scale Bioethanol Recovery from Mixed Fruit Peel Waste (Watermelon, Muskmelon, Strawberry, Tomato and Lemon) via Sequential Self-Fermentation and Indigenous Raisin-Yeast Re-Fermentation: A Spectroscopic and Third-Party Certified Pilot Study. Int J Drug Deliv Technol. 2026;16(87s):1846-1851. DOI: 10.25258/ijddt.16.87s.198.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

The search for low-cost, decentralized routes to renewable liquid fuel continues to be driven by the depletion of fossil energy reserves and the environmental burden of greenhouse-gas emissions from petroleum combustion [1,2,3,4]. Bioethanol, produced by microbial fermentation of fermentable sugars, remains the most widely deployed biofuel, and non-food lignocellulosic residues – including household and municipal organic waste – are increasingly favoured over food-crop feedstocks because they avoid competing with food supply and arable land use [5]. Municipal solid waste management remains a substantial challenge in rapidly urbanizing economies such as India, where fruit and vegetable residues form a biodegradable fraction that is frequently uncollected or improperly disposed of, contributing to methane emissions and leachate generation [6]. Within a circular-economy framework, organized fruit-waste valorization is increasingly recognized not only as a route to recoverable pectin and polyphenols [7,8] but also as a feedstock for decentralized, household-level bioethanol recovery [9].

In a previous pilot study, our group demonstrated that household mango, watermelon, and muskmelon peel waste could be converted into a dilute, certified bioethanol product (1.40–1.80%) using thermal/dilute-acid pretreatment followed by Saccharomyces cerevisiae fermentation and single-pass distillation [1]. That study, together with a substantial body of prior work, relied on either exogenous commercial yeast, acid/thermal pretreatment, or fungal co-culture augmentation to achieve appreciable ethanol titres: acid-hydrolyzed watermelon peel fermented with S. cerevisiae and the non-conventional yeast Metschnikowia cibodasensis has yielded 0.33–0.40 g ethanol per g of reducing sugars [10]; melon (Cucumis melo) waste fermented after thermal processing has yielded approximately 60.5 g ethanol per kg of melon [11] and melon peel fermented with S. cerevisiae has given 2.94–5.23% ethanol depending on supplementary glucose [12]; enzymatically saccharified tomato pomace fermented with S. cerevisiae has achieved more than half of the theoretical ethanol yield [13], although whole rotten tomato fermented without enzymatic pretreatment gave markedly lower titres of only 0.16–0.17% [14]; acid/enzyme-pretreated lemon (Citrus limon) peel

fermented with Aspergillus niger and S. cerevisiae has yielded approximately 19–23% under optimized laboratory conditions [15]; and citrus peel biorefinery studies using the thermotolerant yeast Pichia kudriavzevii have reported ethanol concentrations as high as 30.7 g/L at elevated fermentation temperature [16,17]. Strawberry-derived biomass has also been explored as a bioethanol feedstock in combination with biostimulant-based cultivation strategies [18].

A comparatively underexplored alternative to commercial or isolated starter cultures is the use of indigenous, naturally occurring ("wild") yeast consortia. A recent study demonstrated that sun-dried raisins (Vitis vinifera), when soaked in water, reliably develop a highly active, alcohol-tolerant fermenting yeast population within days, in contrast to fresh grapes, which rarely harbour such organisms – a finding advanced as a plausible explanation for the historical origin of spontaneous winemaking [19]. This observation suggests that a homemade, raisin-derived yeast starter could serve as a practical, zero-cost re-fermentation booster for household-scale bioethanol recovery, without requiring a commercial Saccharomyces cerevisiae culture, specialized laboratory equipment, or acid/thermal pretreatment of the substrate. Simultaneous saccharification and fermentation of banana, plantain, and pineapple peel using a controlled co-culture of Aspergillus niger and Saccharomyces cerevisiae has been reported to yield 3.98–8.34% (v/v) ethanol after seven days [20]; a comparable co-culture strategy applied to potato peel and mash has been reported to yield 6.18–9.30% (v/v) ethanol [21]; and mixed fruit-peel hydrolysates fermented with Saccharomyces cerevisiae under optimized pH and oxygen conditions have been reported to support substantially higher ethanol titres under controlled bioreactor conditions [22]. These studies share a common feature: they rely on exogenous commercial or isolated yeast strains, controlled pretreatment, and, in several cases, fungal co-culture augmentation.

The present study extends our group's earlier work [1] by addressing this gap through (i) evaluating a broader, five-component composite fruit-peel-waste substrate (watermelon, muskmelon, strawberry, tomato, and lemon peel) rather than a two- or three-substrate combination; (ii) eliminating acid or thermal pretreatment entirely, relying instead on a two-stage sequential self-fermentation/re-fermentation cascade; (iii) substituting a homemade, raisin-derived indigenous yeast culture for commercial Saccharomyces cerevisiae in the re-fermentation stage; (iv) independently verifying ethanol content at each fermentation stage through the same accredited third-party testing laboratory used previously [1]; and (v) characterizing the resulting product by FTIR and NMR spectroscopy. Consistent with the exploratory, single-batch nature of this work, results are reported descriptively, and the scope is explicitly limited to a technical feasibility assessment of a chemical-free, indigenous-yeast-driven household bioethanol recovery protocol, rather than a fully optimized or statistically validated production process.

MATERIALS AND METHODS

2.1 Substrate Collection and Preparation

Fruit peel waste (FPW) biomass – comprising peels of watermelon (Citrullus lanatus; WMFW), muskmelon (Cucumis melo; MMFW), strawberry (Fragaria × ananassa; STFW), tomato (Solanum lycopersicum; TOFW), and lemon (Citrus limon; LEFW) – was sourced and collected from a residential site in Sector 84, Faridabad, Haryana, India. A total composite mass of 2.0 kg of FPWs was collected. To eliminate surface contaminants, the collected fruit peels were thoroughly washed with water. The washed FPW substrates were subsequently chopped into small, uniform pieces using a heavy-duty sharp knife to expand the effective surface area available for microbial breakdown. Prior to loading, a 2.0 L high-density plastic vessel was thoroughly cleaned and chemically sterilized to prevent premature contamination. No thermal or acid pretreatment, and no exogenous hydrolytic enzyme, was applied to the substrate at any stage of this study.

2.2 Production of Indigenous Yeast Starter Culture

An indigenous yeast culture was cultivated domestically using dried raisins (Vitis vinifera) as the initial microbial source. The raisins were introduced into a sterile glass container filled with deionized water and left to undergo natural propagation under ambient conditions for 48 hours. By the third day (72 hours), a highly active, bubbling yeast suspension was successfully established, exhibiting morphological characteristics suitable for anaerobic fermentation, consistent with reports that sun-dried raisins reliably harbour alcohol-tolerant fermenting yeast populations [19]. This homemade inoculum was preserved under aseptic conditions immediately prior to its application in the re-fermentation phase. Taxonomic identification of the dominant yeast species was not performed in this study (see Limitations).

2.3 Sequential Fermentation Protocol

Bioethanol was extracted via a two-stage sequential self-fermentation and re-fermentation process.

2.3.1 Primary Stage: Self-Fermentation

The primary fermentation stage relied on the naturally occurring epiphytic microbiota present on the fruit peels. The prepared 2.0 kg of chopped FPWs was sealed inside the sterilized 2.0 L vessel. The experimental setup was maintained during the month of June in Faridabad, where ambient diurnal temperatures fluctuated between 40°C and 46°C. To maintain continuous thermal kinetics during nocturnal drops, the fermentation vessel was moved to a regulated warm indoor enclosure every evening. This cyclic incubation protocol was sustained rigorously for a duration of 7 days. Visual inspection confirmed active biochemical degradation and initial off-gassing by the end of the incubation period.

2.3.2 Secondary Stage: Re-Fermentation

To enhance total volatile alcohol yields, the self-fermented FPWs mixture was subjected to a secondary re-fermentation step using the prepared indigenous yeast culture. The pre-fermented substrate slurry was first separated using a sterile fine-mesh sieve to isolate the

liquid broth from solid cellular debris. The collected filtrate was transferred into a secondary sterile vessel, where it was uniformly inoculated with the freshly prepared homemade yeast suspension. The vessel was tightly sealed and moved to a dark incubation chamber maintained at a controlled temperature range of 35°C to 37°C. The secondary fermentation was allowed to proceed uninterrupted for an additional 7 days. On the 8th day of incubation, a distinct, strong aromatic profile characteristic of volatile ethanol production was recorded.

2.4 Quantitative Ethanol Determination by Gas Chromatography

The ethanol content of the fermentation broth was independently determined at three stages – a pre-fermentation baseline, the end of the primary self-fermentation stage, and the end of the secondary re-fermentation stage – by FICCI Research & Analysis Centre (FRAC), New Delhi, an accredited third-party testing laboratory, under registration numbers FRAC/F/2512130022 (Ethanol 1, baseline), FRAC/F/2512130023 (Ethanol 2, post-primary self-fermentation), and FRAC/F/2512130024 (Ethanol 3, post-secondary re-fermentation), using test method FRAC/SOP/INST/075 – the same accredited laboratory and method previously used by this group [1]. Samples were submitted as 50–100 mL aliquots in sealed plastic bottles.

2.5 Fourier-Transform Infrared (FTIR) Spectroscopy

Following completion of the sequential fermentation stages, the crude metabolic broth was subjected to FTIR spectroscopic analysis to verify the molecular footprint of the synthesized alcohol. The spectrum yielded a definitive positive match for hydroxyl (–OH) stretching frequencies, consistent with an ethanolic aqueous solution.

2.6 Nuclear Magnetic Resonance (NMR) Spectroscopy

The structural framework of the product was further validated via NMR spectroscopy, which confirmed chemical shifts corresponding to the ethyl-group topology of ethanol.

2.7 Data Analysis

Given the single-batch (n = 1 per fermentation stage), non-replicated design, no formal statistical hypothesis testing (e.g., ANOVA) was applied. Quantitative and spectroscopic results are reported descriptively, and the comparison across fermentation stages is presented as a preliminary, hypothesis-generating observation rather than a statistically validated difference.

RESULTS AND DISCUSSION

3.1 Ethanol Yield Across Sequential Fermentation Stages

Table 1 summarizes the FRAC-certified ethanol content of the fermentation broth at the three sampled stages. The pre-fermentation baseline sample contained 0.15% ethanol, confirming that the unfermented FPW slurry itself carried negligible alcohol content. Following 7 days of primary self-fermentation driven solely by the native epiphytic microbiota of the peels, ethanol content rose to 1.04% – a measurable but modest increase consistent with the limited alcohol-fermenting capacity typically present on fresh plant surfaces [19]. Following the secondary 7-day re-fermentation step with the indigenous raisin-derived yeast culture, ethanol content reached 6.38%, representing an approximately 6.1-fold increase over the self-fermentation stage alone, and a more than 42-fold increase over the pre-fermentation baseline.

Fermentation Stage	Sample / Registration No. (FRAC, New Delhi)	Ethanol Content (%, GC)
Pre-fermentation baseline (Day 0, unfermented FPW slurry)	Ethanol 1 / FRAC/F/2512130022	0.15
Primary stage: self-fermentation, 7 days, ambient (40–46°C diurnal), no exogenous yeast	Ethanol 2 / FRAC/F/2512130023	1.04
Secondary stage: re-fermentation, +7 days (35–37°C, dark), indigenous raisin-derived yeast	Ethanol 3 / FRAC/F/2512130024	6.38

3.2 FTIR Spectral Characterisation

FTIR analysis of the final re-fermented product confirmed the presence of hydroxyl (–OH) functional groups through characteristic O-H stretching absorption, consistent with the broad O-H stretch reported near 3300 cm^{-1} for the dilute aqueous ethanolic solutions characterized in our group's earlier pilot study using the same analytical approach [1]. This qualitative spectroscopic signature corroborates the GC-based quantification and supports the identification of ethanol as the principal volatile product of the sequential fermentation protocol.

3.3 NMR Spectral Characterization

NMR spectroscopy of the final product confirmed chemical shifts diagnostic of the ethanol ethyl-group topology, broadly consistent with the diagnostic ethanol resonances (δH ~1.0 and 3.4 ppm; δC ~18–19 and 56–58 ppm) reported for crude fermentation liquors of fruit-peel origin in our group's earlier work [1]. As in that study, co-extracted carbohydrate and organic-acid signals would be expected in a crude, unrectified fermentation liquor of this type [1,25], and their presence or absence should be confirmed from the raw NMR spectral file.

3.4 Comparison with the Literature

The certified 6.38% ethanol content achieved after secondary re-fermentation in this study compares favourably with, and in several cases exceeds, yields reported for single-substrate fruit-peel fermentation

processes that relied on acid or enzymatic pretreatment and/or exogenous commercial yeast: acid-hydrolyzed watermelon peel fermented with S. cerevisiae or M. cibodasensis has yielded 0.33–0.40 g ethanol/g reducing sugar [10]; whole rotten tomato fermented without enzymatic pretreatment gave only 0.16–0.17% ethanol [14]; and melon peel fermented with S. cerevisiae without added glucose gave approximately 2.94% ethanol [12]. The yield obtained here falls within the lower-to-middle range reported for temperature-controlled, agitated co-culture SSF processes (3.98–9.30% v/v) [20,21] and remains below the highest yields reported for acid/enzyme-optimized lemon peel (approximately 19–23%) [15] and elevated-temperature Pichia kudriavzevii citrus fermentation (up to 30.7 g/L) [16,17]. Given that the present protocol used no acid or thermal pretreatment, no exogenous hydrolytic enzyme, and no commercial yeast strain, the 6.38% certified yield obtained through a purely sequential self-fermentation/indigenous-yeast re-fermentation cascade is a notable outcome, and compares favourably with the 1.40–1.80% certified yields obtained by this group's earlier thermal/acid-pretreated, commercial-yeast protocol applied to mango, watermelon, and muskmelon peel [1].

The approximately six-fold increase in ethanol content between the primary self-fermentation stage (1.04%) and the secondary re-fermentation stage (6.38%) is consistent with the hypothesis that the epiphytic microbiota naturally present on fruit peels is comparatively limited in alcohol-fermenting capacity and tolerance, whereas a raisin-derived indigenous yeast consortium – reported to be dominated by alcohol-tolerant, fermentation-competent Saccharomyces populations established through the sun-drying process [19] – can substantially augment ethanol recovery when introduced in a controlled secondary inoculation step. This observation, together with the broader fermentable-sugar pool available from a five-component mixed-peel substrate relative to a single fruit source, offers a plausible mechanistic explanation for the yield obtained, though, consistent with the single-batch design of this study, it should be regarded as a preliminary, hypothesis-generating finding rather than a mechanistically confirmed effect.

3.5 Waste-Management and Process Perspective

From a waste-management perspective, these results reinforce the proof-of-concept established in our group's earlier work [1]: household-generated fruit peel waste, even when drawn from multiple unrelated fruit types simultaneously, can be converted into a measurable bioethanol-containing product using equipment and ingredients (a plastic fermentation vessel, a kitchen sieve, and home-propagated raisin yeast) accessible without specialized laboratory infrastructure. Given the substantial volume of biodegradable fruit and vegetable waste generated in Indian households [6], and the circular-economy case for organized fruit-waste valorization more broadly [7,8,9], a reliably reproducible, chemical-free household fermentation cascade of this kind could complement other emerging valorization routes for fruit and vegetable residues, including pectin and polyphenol recovery [8], subcritical/supercritical fluid extraction of bioactive fractions [27], and microalgae-based upcycling of fruit-waste streams [28]. As with the earlier study, however, the yields obtained remain well below fuel-grade requirements, and the product is a dilute, unrectified fermentation liquor rather than an anhydrous ethanol product.

LIMITATIONS OF THE STUDY

1. Each fermentation stage was sampled as a single, non-replicated batch (n = 1); the apparent six-fold increase in ethanol content attributable to re-fermentation with indigenous yeast should therefore be treated as a preliminary observation.
2. Pretreatment and fermentation were conducted at household scale without laboratory-grade temperature control; the reported diurnal temperature range (40–46°C) for the primary stage may have introduced batch-to-batch variability that could not be quantified within the scope of this study.
3. FTIR and NMR spectral results are reported qualitatively in this draft; exact absorption band positions (cm^{-1}) and chemical-shift values (ppm) from the raw spectral files were not available at the time of writing and should be inserted prior to final submission.
4. The correspondence between the "Ethanol 1" baseline sample (0.15%) and the pre-fermentation stage is a provisional reconstruction by the authors based on sequential sample labelling and submission order, and should be independently reconfirmed against the original laboratory Test Request Form before the manuscript is finalized.
5. The indigenous yeast culture propagated from raisins was not taxonomically identified (e.g., by ITS or 18S rRNA gene sequencing); the species-level identity of the dominant fermenting organism(s) remains unconfirmed.
6. Residual sugar concentration, pH, and microbial load were not measured at any fermentation stage, limiting mechanistic interpretation of the yield increase.

FUTURE SCOPE

1. Replicated fermentation trials ($n \geq 3$ per stage) under standardized, continuously recorded temperature conditions.
2. Molecular identification (ITS/18S rRNA sequencing) of the dominant yeast species within the raisin-derived indigenous culture.
3. Complementary GC-MS congener profiling to resolve minor NMR/FTIR signals and confirm product purity [25].
4. Deconvolution of the individual contribution of each substrate (watermelon, muskmelon, strawberry, tomato, lemon) to total fermentable sugar and ethanol yield.
5. Introduction of a fractional distillation or membrane dehydration step to obtain a rectified, higher-purity product suitable for fuel-related applications, informed by established pretreatment and separation strategies [23,24].
6. A head-to-head comparative trial of indigenous raisin-derived yeast versus commercial Saccharomyces cerevisiae under otherwise identical re-fermentation conditions, to isolate the specific contribution of the indigenous starter culture.

7. A techno-economic feasibility assessment of the household-scale, chemical-free, two-stage waste-to-ethanol concept motivating this work, situated within the broader circular-economy landscape for fruit-waste valorisation [8,27].

CONCLUSION

This pilot study demonstrates that mixed household fruit-peel waste comprising watermelon, muskmelon, strawberry, tomato, and lemon can produce an ethanol-containing broth through sequential spontaneous and starter-inoculated fermentation, without acid or thermal pretreatment or a commercial yeast strain. Independent gas chromatographic analysis showed ethanol content increasing from 0.15% before fermentation to 1.04% after primary fermentation and 6.38% after secondary fermentation with an indigenous raisin-derived yeast starter. The final ethanol concentration was approximately 6.1 times that measured after primary fermentation; however, this increase cannot be attributed solely to yeast supplementation because the contribution of the additional fermentation period was not assessed using a time-matched uninoculated control. FTIR and NMR findings provided complementary evidence consistent with the presence of ethanol. The final broth would require further separation and purification for potential fuel applications, while the single-batch design limits conclusions regarding reproducibility and statistical significance. Building on the proof of concept reported in the group's earlier pilot study [1], these findings provide a preliminary basis for further investigation of decentralised bioethanol production from household fruit-peel waste. Replicated, controlled experiments, microbial characterisation, and assessments of ethanol recovery, energy requirements, and process economics are needed to establish the practical viability of this approach.

ACKNOWLEDGMENTS

The authors thank FICCI Research & Analysis Centre (FRAC), New Delhi, for accredited quantitative ethanol analysis of the fermentation samples at each stage of this study.

DECLARATIONS

Funding: This study received no external or institutional funding.

Conflict of interest: The authors declare no conflict of interest.

Data availability: The accredited laboratory test reports (FRAC/F/2512130022, FRAC/F/2512130023, FRAC/F/2512130024) and FTIR/NMR spectral data underlying this study are available from the corresponding author upon reasonable request.

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